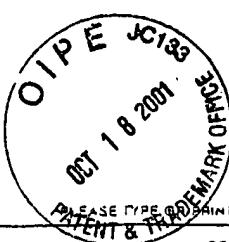


ANIMAL USAGE FORM



AUF 1413

1. PRINCIPAL INVESTIGATOR

WAYNE A. BORDER, M.D.

OFFICE PHONE

226

HOME/EMERGENCY PHONE

(714) 770-4602

2. OTHER INVESTIGATOR

LUCIA LANGUINO, Ph.D.

230

539-0609

3. SENIOR TECHNICIAN

4. PROJECT TITLE

ANTI-HUMAN TGF β CYCLIZED PEPTIDE

5. GRANT NUMBER, IF ANY

250200

NEW

RENEWAL

PILOT

PROJECT NUMBER

X

6. START DATE

END DATE

QUANTITY:

MICE

RATS

RABBITS

GP1

OTHER (SPECIFY)

2

7. PROJECT GOALS (SEE INSTRUCTIONS)

To produce quantities of anti-human TGF β cyclized peptide for use in kidney disease research.

8. RATIONALE (SEE INSTRUCTIONS)

Rabbits produce high quality antiserum which can be used for identification of human TGF β in tissue samples and in vitro assays to study progression of kidney injury.

9. DESCRIBE USE OF ANIMALS (SEE INSTRUCTIONS)

All injections/bleedings to be performed by animal care facility personnel.

1. Pre-bleeding 20 ml from ear vein.
2. Inject 50 μ g TGF β cyclized purified peptide (0.5 ml antigen in PBS + 0.5 ml FCA) subcutaneously in 2 sites.
3. After one month, boost with 125 μ g antigen (0.25 ml antigen in PBS + 0.25 ml incomplete adjuvant) subcutaneously, 2 sites.
4. After 10 days, bleed 50 ml from alternating ear veins 3 times.
5. Repeat steps 3-4 at 4-6 week intervals.

REASONABLE EFFORTS ARE MADE TO MINIMIZE
ANIMAL SUFFERING AND DEATH.
INSTRUCTIONS IN THIS FORM ARE
ANOTHER EXAMPLE OF THE WAY THIS
RESEARCH IS CONDUCTED.

RESEARCH PRACTICAL

10. PAIN LEVEL

A

B

C

(IF B OR C READ INSTRUCTIONS. PROVIDE DESCRIPTION OR JUSTIFICATION HERE)

CONFIDENTIAL

11. SUTURANASIA (SEE INSTRUCTIONS)

DURING PROJECT	<input type="checkbox"/>	METHOD OR TECHNIQUE	CO.	<input type="checkbox"/>	CERV. DISLOC.	<input type="checkbox"/>	RETAIN CARCASSES? YES	<input type="checkbox"/>
END OF PROJECT	<input type="checkbox"/>		CO.	<input type="checkbox"/>	OTHER (SPECIFY)	<input type="checkbox"/>	FOR PH	<input type="checkbox"/>

12. SIGNATURES

PI	WA Border	DATE
AP		
MGR		

U2 05334

ANIMAL PROCEDURE REQUEST

(For procedures to be performed by Animal Facility personnel)
Return this form to the Animal Facility Office.

LJCRF

TODAY'S DATE:

Principal Investigator E. ROSENTHAL

Lucia F. Langstroth

Phone Lab No.

230

Lab No. _____
Phone _____

This procedure relates to the project outlined on Annex 1 usage form No.

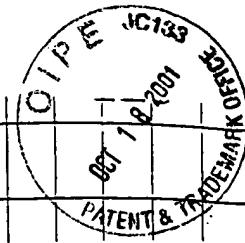
Date or Dates on which this procedure is required (please allow 7 days for scheduling):

1	12/13	188	50 w/	(1f more)	5
2	12/16	188	"	(than 8 pro- cedures are) (required, attach a)	6
3	12/21	188	"	Ent. 1004 (Second form)	7

5

Describe the procedure(s) required:

Vol. of blood to be collected each procedure: ml.





Injection of 2 rabbits with Linear TF-109 peptide
~86-82 from $\text{TG}\bar{\beta}$.

2 " with cyclized TF-109 "

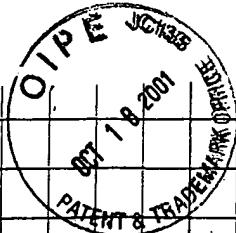
~88-83

~~coupling~~

Procedure: 2 mg / rabbit of each peptide.

- 2 mg peptide were dissolved in 250 μl DDM
(add to the solution to couple the peptide)
- 0.5 mg Hutylenediamine was ~~added~~ ~~coupled~~
(Sigma A1003 : H₂BSA)
- vortex 30"
- Added 250 μl Freud's adjuvant complete
- mixed 1 h with homogenizer
- 0.5 ml solution was injected in each rabbit

Note: Both peptides were difficult to dissolve.
" were not fully purified.



Injection of 2 rabbit linear TGF β peptide

Cyclic =

PG peptide

Procedure

except Friend's incomplete adjuvant was used

For usage: KLH-peptide was already coupled

It is only mixed to F.i. dose, mix 1h 1:5

inject 0.5 ml

Rabbl. 7

n° 1285

1285

g = PG

A.I.F. 1385